VII - CLAIMING

What is claimed is:

1. The procedure for cloning human βA precursor protein gene (human APP gene) based on the reverse transcription (RT) and the polymerase chain reaction (PCR) using the synthesized oligonucleotides (a) for RT, and (b) and (c) for PCR respectively, comprising:

- Isolation of APP-mRNA.

- RT reaction using the synthesized oligonucleotide (a) with the following sequence: 5' GTTACAGCACAG 3' (a).

- RT reaction conditions: 90°C for 2 minutes; 0°C for 1 minute; 25°C for 10 minutes; 42°C for 45 minutes.

- PCR reaction using the synthesized oligonucleotides (b) and (c) with the following sequences:
5' ATGCTGCCCGGTTTGGC 3' (b).
5' CTAGTTCTGCATCTGCTCA 3' (c).

- PCR reaction conditions: Denaturation at 94°C for 1 minutes; annealing at 55°C for 2 minutes; elongation at 72°C for 3 minutes each cycle, for 35 cycles.

2. The procedure for the construction of expression plasmids using the pFastBac™ HTb and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human APP in insect cells, comprising:

2.1. Using the pFastBac™ HTb vector:

- Digesting the pFastBac™ HTb vector with XbaI and HindIII followed by dephosphorylation with calf intestinal alkaline phosphatase.

- Digesting the vectors (1) pCR^R II/APP$_{751}$-cDNA and (2) pCR^R II/APP$_{770}$-cDNA with XbaI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP$_{751}$-cDNA and APP$_{770}$-cDNA.

- Ligating the APP$_{751}$-cDNA and APP$_{770}$-cDNA fragments to the pFastBac™ HTb vectors and introducing the ligation products in INVαF' E. Coli strain.

- Screening for inserts based on the presence of white colonies, as a result of which

the vectors (**3**) pFastBac™ HTb /APP_{751}-cDNA and (**4**) pFastBac™ HTb /APP_{770}-cDNA are selected.

- Introducing the vectors (**3**) and (**4**) in DH10Bac™ E. Coli competent cells.

- Screening for recombinant bacmids in DH10Bac™ E. Coli using blue-white color selection, then verifying the presence of APP-cDNA's inserts in the recombinant bacmids by PCR amplification using the M13 forward (-40) and M13 reverse primers. As a result, the recombinant bacmids (**5**) for vectors (**3**) in DH10Bac™ E. Coli and (**6**) for vector (**4**) in DH10Bac™ E. Coli respectively are selected.

2.2. Using the pBlueBacHis2 A vector:

- Digesting the pBlueBacHis2 A vector with NcoI and HindIII followed by dephosphorylation with calf intestinal phosphatase.

- Digesting the vectors (**3**) pFastBac™ HTb/APP_{751}-cDNA and (**4**) pFastBac™ HTb/APP_{770}-cDNA with NcoI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP_{751}-cDNA and APP_{770}-cDNA.

- Ligating the APP_{751}-cDNA and APP_{770}-cDNA fragments to the pBlueBacHis2 A vectors and introducing the ligation products in INVαF' E. Coli strain.

- Screening for inserts using blue-white color selection, as a result of which the vectors (**7**) pBlueBacHis2 A/APP_{751}-cDNA and (**8**) pBlueBacHis2 A/APP_{770}-cDNA are selected.

3. The procedure for the construction of expression plasmids using the pET-28a (+) transfer vector for the purpose of obtaining human APP in bacteria, comprising:

- Digesting the pET-28a (+) vector with SalI and HindIII followed by dephosphorylation with calf intestinal alkaline phosphatase.

- Digesting the vector (**3**) pFastBac™ HTb/APP_{751}-cDNA and (**4**) pFastBac™ HTb/APP_{770}-cDNA with SalI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP_{751}-cDNA and APP_{770}-cDNA.

- Ligating the APP_{751}-cDNA and APP_{770}-cDNA fragments to the pET-28a (+) vectors and introducing the ligation products in INVαF' E. Coli strain.

- Screening for inserts based on the presence of white colonies, as a result of which the vectors (**9**) pET-28a (+)/APP_{751}-cDNA and (**10**) pET-28a (+)/APP_{770}-cDNA are selected.

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